

U.S. Serial No. 10/045,949

Amendments to the Claims

Please amend the claims as follows:

1. (currently amended) A recombinant fusion protein, comprising:  
a GFP-like chromophore;  
at least one self-multimerization domain; and  
an MHC peptide-presenting moiety.
2. (original) A nucleic acid, comprising: a sequence that encodes the fusion protein of claim 1 or is complementary to a sequence that encodes the fusion protein of claim 1.
3. (original) A recombinant vector, comprising: the nucleic acid of claim 2.
4. (original) The recombinant vector of claim 3, wherein said vector is capable of directing expression of said fusion protein in a host cell.
5. (previously presented) A host cell, said host cell comprising:  
the recombinant vector of claim 4.
6. (original) An intrinsically fluorescent, multimeric protein complex, comprising:  
a plurality of subunits, said subunits having the quaternary formula in said complex of  
 $(F)_n$ ,  
wherein F is a fusion protein according to claim 1 and n is an integer greater than 1.
7. (currently amended) An intrinsically fluorescent, multimeric protein complex, comprising:  
a plurality of subunits, said subunits having the quaternary formula in said complex of

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wherein

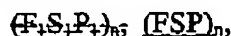
F is a recombinant fusion protein according to claim 1;

S is a soluble protein selected from the group consisting of  $\beta$ 2 microglobulin, class II  $\beta$  MHC peptide-presenting soluble derivatives, and class II  $\alpha$  MHC peptide-presenting soluble derivatives;

S is  $\beta$ 2 microglobulin when F includes a class I  $\alpha$  MHC peptide-presenting moiety, S is a class II  $\beta$  MHC peptide-presenting soluble derivative when F includes a class II  $\alpha$  MHC peptide-presenting moiety, and S is a class II  $\alpha$  MHC peptide presenting soluble derivative when F includes a class II  $\beta$  MHC peptide-presenting moiety; and  
n is an integer greater than 1.

8. (currently amended) An intrinsically fluorescent, multimeric protein complex for labeling T lymphocytes according to the specificity of their antigen receptors, comprising:

a plurality of subunits, said subunits having the quaternary formula in said complex of



wherein

F is a recombinant fusion protein according to claim 1;

S is a soluble protein selected from the group consisting of  $\beta$ 2 microglobulin, class II  $\beta$  MHC peptide-presenting soluble derivatives, and class II  $\alpha$  MHC peptide-presenting soluble derivatives;

S is  $\beta$ 2 microglobulin when F includes a class I  $\alpha$  MHC peptide-presenting moiety, S is a class II  $\beta$  MHC peptide-presenting soluble derivative when F includes a class II  $\alpha$  MHC peptide-presenting moiety, and S is a class II  $\alpha$  MHC peptide presenting soluble derivative when F includes a class II  $\beta$  MHC peptide-presenting moiety;

P is a peptide antigen; and

n is an integer greater than 1.

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9. (currently amended) A recombinant fusion protein, comprising:  
a means for fluorescing, said means encoded entirely within the amino acid sequence of said protein;  
a means for self-multimerizing; and  
a means that is capable of contributing to presentation of a peptide antigen to an MHC-restricted T lymphocyte.

10. (currently amended) An intrinsically fluorescent, multimeric protein complex for labeling T lymphocytes according to the specificity of their antigen receptors, comprising:

a means for fluorescing, ~~said means being~~ encoded entirely within an amino acid sequence of at least one subunit of said multimeric complex;

a means for self-multimerizing encoded entirely within an amino acid sequence of at least one subunit of said multimeric complex; and

a means for binding to a T lymphocyte according to the specificity of its antigen receptor.

11. (original) A method for detectably labeling a T lymphocyte according to the specificity of its antigen receptor, the method comprising:

contacting said T lymphocyte with an intrinsically fluorescent multimeric complex according to claim 8, the antigen receptor of said T lymphocyte being specific for the peptide antigen and the MHC presenting domains of said complex, for a time and under conditions sufficient to permit detectable binding of said complex to said T lymphocyte.

12. (original) A method for detecting, in a sample of cells, T lymphocytes that are specific for a chosen antigen, comprising:

contacting said sample with an intrinsically fluorescent multimeric complex according to claim 8, wherein the peptide antigen of the complex is the chosen antigen and the MHC presenting domains of the complex are those for which the T lymphocytes desired to be detected will be restricted, for a time and under conditions sufficient to

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permit detectable binding of said complex to T lymphocytes specific for said chosen antigen; and then

detecting specific T lymphocytes in said sample by the fluorescence of the complex bound thereto.

13. (original) The method of 12, further comprising: enumerating the antigen-specific T lymphocytes so detected.

14. (original) The method of claim 12, further comprising:

contacting said sample with at least one fluorophore-conjugated antibody, said antibody selected from the group consisting of pan-T antibodies and T cell subsetting antibodies;

detecting cell-bound fluorescence of the multimeric fluorescent complex; and

detecting cell-bound fluorescence from the at least one fluorophore-conjugated antibody.

15. (original) The method of claim 12, further comprising:

contacting said sample with at least one fluorophore-conjugated antibody specific for a T cell activation antigen, and then

detecting activated specific T lymphocytes in said sample by the fluorescence of the multimeric fluorescent complex and at least one fluorophore-conjugated antibody bound thereto.

16. (original) A method for enriching a sample in T lymphocytes that are specific for a chosen antigen, comprising:

contacting said sample with an intrinsically fluorescent multimeric complex according to claim 8, wherein the peptide antigen of said complex is said chosen antigen, for a time and under conditions sufficient to permit detectable binding of said complex to T lymphocytes specific for said chosen antigen; and then

enriching for said specific T lymphocytes based upon the fluorescence of the complex bound thereto.

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17. (original) A method for depleting a sample of T lymphocytes that are specific for a chosen antigen, comprising:

contacting said sample with an intrinsically fluorescent multimeric complex according to claim 8, wherein the peptide antigen of said complex is said chosen antigen, for a time and under conditions sufficient to permit detectable binding of said complex to T lymphocytes specific for said chosen antigen; and then

depleting said sample of specific T lymphocytes based upon the fluorescence of the complex bound thereto.

18. (original) A kit comprising, as separate compositions:

the multimeric complex of claim 7; and

a peptide antigen.

19. (original) The kit of claim 18, further comprising:

a fluorophore-conjugated antibody selected from the group consisting of pan-T antibody and T cell subsetting antibody.

20. (original) The kit of claim 18 or claim 19, further comprising:

a fluorophore-conjugated antibody specific for a T cell activation antigen.

21. (original) A kit comprising, as separate compositions:

the multimeric complex of claim 8; and

a fluorophore-conjugated antibody selected from the group consisting of pan-T antibody and T cell subsetting antibody.

22. (original) The kit of claim 21, further comprising:

a fluorophore-conjugated antibody specific for a T cell activation antigen.

23. (original) The kit of either of claims 18 or 21, further comprising:

a red blood cell lysing agent.

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24. (original) The recombinant fusion protein of claim 1, further comprising a flexible peptide spacer.